

In re Application of:
Cope et al.
Application No.: 10/047,253
Filed: January 14, 2002
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ATTORNEY DOCKET NO.: CIT1510-4

In the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Upon entry of the present amendment, the claims will stand as follows:

Please amend claims 32, 34, 41 and 74 as follows:

Claims 1-31 (Cancelled)

32. (Currently Amended) A method of-identifying an agent that affects isopeptidase activity of a polypeptide comprising:

incubating a test agent with a polypeptide comprising a JAb1/Mpn domain Metalloenzyme (JAM) domain consisting essentially of an amino acid sequence of HXHXXXXXXXXXXD, wherein H is histidine, D is aspartate, and X is any amino acid,
wherein the isopeptidase activity deconjugates a modifier protein from a target protein, ~~wherein the modifier protein is conjugated to the target protein via~~ by cleaving a peptide bond formed
between the carboxy terminus of the modifier protein and a free amino group of the target protein in proximity to a metal ion, ~~the method comprising: ; and~~

determining the isopeptidase activity of the polypeptide in the presence and absence of the test agent, wherein a difference in the isopeptidase activity in the presence versus the absence of the test agent is indicative of an agent that affects isopeptidase activity of the polypeptide.

33. (Original) The method of claim 32, wherein the JAM domain consists essentially of an amino acid sequence of GW(Y/I)H(S/T)HPXXXXXXXXSXXD (SEQ ID NO. 2), wherein G is glycine, W is tryptophan, Y is tyrosine, I is isoleucine, H is histidine, S is serine, T is threonine, P is proline, D is aspartate, X is any amino acid, Y/I is either Y or I, and S/T is either S or T.

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34. (Currently Amended) The method of claim 32, wherein the target protein is a cullin protein.

35. (Original) The method of claim 34, wherein the target protein is Cul1, Cul2, Cul3, Cul4A, Cul4B, or Cul5.

36. (Original) The method of claim 32, wherein the target protein has ubiquitin ligase activity.

37. (Original) The method of claim 32, wherein the target protein is part of a protein complex having ubiquitin ligase activity.

38. (Withdrawn) The method of claim 32, wherein the modifier protein is NEDD8, UBL1, SMT3H2, SMT3H1, APG12, FAT10, Fau, UCRP, URM1, or UBL5.

39. (Original) The method of claim 32, wherein the polypeptide is a polypeptide complex of COP9/signalosome.

40. (Withdrawn) The method of claim 32, wherein the polypeptide is AMSH, AMSH1, or AMSH2.

41. (Currently Amended) The method of claim 32, wherein an increase in a ~~test agent decreasing~~ the amount of the target protein not conjugated to the modifier protein is indicative of an agent that decreases increases deconjugation of the modifier protein from the target protein.

42. (Original) The method of claim 32, wherein the target protein has the activity of peroxidase, alkaline phosphatase, or luciferase.

43. (Original) The method of claim 32, wherein the target protein is a fluorescent protein.

44. (Original) The method of claim 43, wherein the fluorescent protein is selected from the group consisting of green fluorescent protein, yellow fluorescent protein, cyan fluorescent protein and dsRed.

45. (Original) The method of claim 43, wherein the target protein is a fluorescent protein via chemical modification.

46. (Original) The method of claim 32, wherein the target protein causes production of a detectable signal upon deconjugation from the modifier protein.

47. (Original) The method of claim 32, wherein the polypeptide is a polypeptide complex of 26S proteasome.

48. (Original) The method of claim 32, wherein the polypeptide is a polypeptide complex of 26S proteasome and the modifier protein is an ubiquitin.

49. (Original) The method of claim 47, wherein the incubation is conducted in the presence and absence of the test agent, the target protein, the 26S proteasome, and a 20S inhibitor.

50. (Original) The method of claim 47, wherein the incubation is conducted in the presence and absence of the test agent, the target protein, the 26S proteasome, a 20S inhibitor, and ATP.

51. (Original) The method of claim 50, wherein the incubation further includes an inhibitor of deubiquitination by an ubiquitin isopeptidase.
52. (Original) The method of claim 47, wherein the target protein not conjugated to the modifier protein is not degraded.
53. (Original) The method of claim 47, wherein the target protein is Sic1.
54. (Original) The method of claim 47, wherein the 26S proteasome is purified from *S. cerevisiae*.
55. (Original) The method of claim 47, wherein the 26S proteasome is purified from eukaryotic cells.
56. (Original) The method of claim 47, wherein the 26S proteasome is purified from human cells.
57. (Original) The method of claim 32, wherein the test agent is a member of a compound library selected from the group consisting of hydroxamate compound library, reverse hydroxamate compound library, carboxylate compound library, thiol compound library, and phosphonate compound library.

Claims 58-71 (Cancelled)

72. (Previously Presented) The method of claim 32, wherein the polypeptide comprising the JAM domain comprises Rpn11.

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73. (Previously Presented) The method of claim 72, wherein the polypeptide comprising the JAM domain is Rpn11.

74. (Currently Amended) The method of claim 72, wherein the method further comprises carrying out the incubation in the presence of an inhibitor of degradation of the target protein.

75. (Previously Presented) The method of claim 32, further comprising after the incubation, determining whether the modifier protein remains conjugated to the target protein via a peptide bond formed between the carboxy terminus of the modifier protein and a free amino group of the target protein.

76. (Previously Presented) The method of claim 32, wherein the polypeptide comprising the JAM domain has isopeptidase activity.